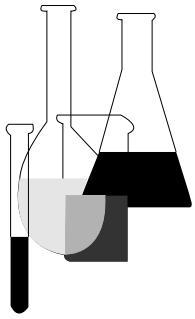
United States
Environmental Protection
Agency

Prevention, Pesticides
and Toxic Substances
(7101)

EPA 712–C–96–157
April 1996

Ecological Effects Test Guidelines

OPPTS 850.4450 Aquatic Plants Field Study, Tier III



“Public Draft”

INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Public Draft Access Information: This draft guideline is part of a series of related harmonized guidelines that need to be considered as a unit. *For copies:* These guidelines are available electronically from the EPA Public Access Gopher (gopher.epa.gov) under the heading ‘‘Environmental Test Methods and Guidelines’’ or in paper by contacting the OPP Public Docket at (703) 305–5805 or by e-mail: guidelines@epamail.epa.gov.

To Submit Comments: Interested persons are invited to submit comments. By mail: Public Docket and Freedom of Information Section, Office of Pesticide Programs, Field Operations Division (7506C), Environmental Protection Agency, 401 M St. SW., Washington, DC 20460. In person: bring to: Rm. 1132, Crystal Mall #2, 1921 Jefferson Davis Highway, Arlington, VA. Comments may also be submitted electronically by sending electronic mail (e-mail) to: guidelines@epamail.epa.gov.

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on *The Federal Bulletin Board*. By modem dial 202–512–1387, telnet and ftp: fedbbs.access.gpo.gov (IP 162.140.64.19), or call 202–512–0135 for disks or paper copies. This guideline is also available electronically in ASCII and PDF (portable document format) from the EPA Public Access Gopher (gopher.epa.gov) under the heading ‘‘Environmental Test Methods and Guidelines.’’

OPPTS 850.4450 Aquatic plants field study, Tier III.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*).

(2) **Background.** The source material used in developing this harmonized OPPTS test guideline is OPP 124–2 Aquatic Field Testing (Pesticide Assessment Guidelines, Subdivision J—Hazard Evaluation; Nontarget Plants) EPA report 540/09-82-020, 1982.

(3) **Test objective.** This guideline should be used in conjunction with OPPTS guideline 850.4000, Background—Nontarget plant testing, which provides general information and overall guidance for the nontarget plants test guidelines.

(i) **General.** (A) This guideline should be used in conjunction with OPPTS guideline 850.4000, Background—Nontarget plant testing, which provides general information and overall guidance for Group D of the 850 series. Aquatic field testing studies (Tier III) are designed to provide phytotoxicity data on a pesticide. These phytotoxicity data are needed to evaluate the effect of the level of pesticide exposure to nontarget aquatic plants and to assess the impact of pesticides on endangered and threatened plants as noted under the Endangered Species Act. Where a phytotoxic effect is noted in one or more plants, further field aquatic field testing studies may be required. These studies are required by 40 CFR 158.150 to support the registration of any pesticide intended for outdoor use under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), as amended.

(B) Pesticides with outdoor use patterns that do not readily release the pesticide to the environment do not have to be evaluated using this phytotoxicity test. These use patterns include tree injection, subsurface soil applications, recapture systems, and wick applications. However, if any of these use patterns do readily expose nontarget plants to the pesticide, the pesticide phytotoxicity potential may need to be evaluated.

(ii) **Objective of the aquatic field testing Tier III test.** (A) The objective of the Tier III aquatic field testing study is to determine if a pesticide exerts a detrimental effect to plants during critical stages in their development. The test is performed on species from a cross-section of the nontarget aquatic plant population. It is also performed under natural conditions and in the environment in which the pesticide is to be applied. By this procedure, direct assessments can be made as to the potential phytotoxicity of the pesticide. This is a multiple dose test designed to evaluate the phytotoxic effects of the pesticide over as wide a range of anticipated pesticide quantities as may be found in the environment.

(B) The Tier III aquatic nontarget plant phytotoxicity field studies are required if greater than 50 percent adverse effects on plant growth

for aquatic plants are expected to occur when the product is used as directed by the label (the estimated environmental concentration exceeds the EC50 aquatic values). The Tier III tests are expected to provide information on detrimental effects to plants during critical stages of development. The typical end-use product (TEP) is used to assess effects on a broader range of nontarget plant species in a number of geographic areas.

(b) **Test standards.** In addition to the general test standards set forth in OPPTS 850.4000, the test standards for this guideline are the same as those in OPPTS 850.4400, with the following modifications:

(1) **Test substance.** The test substance is to be the end-use product or a representative end-use product from the same major formulation category for that general use pattern such as wettable powders, emulsifiable concentrates, and granulars. (If the manufacturing-use product is usually formulated into end-use products comprising two or more major formulation categories, a separate study must be performed with a typical end-use product for each category.) The test substance should contain the highest percentage of active ingredient (AI) and/or be the most widely used (total pounds AI).

(2) **Application levels.** The dosages tested should be the same as those specified in the Tier II aquatic test standards in guideline OPPTS 850.4400.

(3) **Species.** (i) Aquatic plant representatives of the following plant groups are to be tested:

(A) *Dicotyledonae* (dicots), one representative.

(B) *Monocotyledonae* (monocots), representatives of three families.

(C) Vascular *Cryptogamae* (ferns and allies), representatives of three families.

(D) Algae (including *Cyanophyta*), a representative of each division.

(E) *Bryophyta* (mosses) or *Hepatophyta* (liverworts), one representative (not required for true aquatic use patterns, rather for wetland use patterns).

(ii) Plant species used for testing Tiers I and II can be used to satisfy the monocot and dicot test plant requirements of this guideline.

(iii) Additional plant species may be required if the general selectivity of the pesticide cannot be readily identified.

(4) **Environmental conditions.** (i) Plants may be grown in either native soil, water, or other substrate of similar nature to that of the indigenous area or under other conditions similar to the natural habitat.

(ii) Reduction of light intensity by natural or constructed light shade may be necessary to simulate the reduced light intensities found with certain plant communities such as deeply submerged sites or shaded waters.

(iii) Other natural conditions should also be maintained where plants are removed from their natural habitat. Soil, water, and air temperatures should approximate those of the natural habitat. For estuarine and marine habitats, the following conditions should, to the extent possible, simulate the natural environment: Tidal action, water turbidity, flow rates, salinity, and degree of exposure.

(iv) Tests should be performed either in enclosed, controlled areas of a lake, pond, or swamp, or in large water cultures such as aquaria or plastic wash tubs. Tests are not to be performed in dynamic or flowing water where the release of the chemical cannot be contained or its escape prevented.

(v) The field studies should be conducted using acceptable protocols as may be found in paragraph (e)(5) of this guideline Truelove, B., 1977, Research Methods in Weed Science, 2nd Ed. Southern Weed Science Society, Auburn Printing Inc., Auburn, AL or a protocol with prior approval of the Agency.

(5) **Duration.** The test duration should be of sufficient length to assess multiple applications directed by the label. Observations should continue for the entire life cycle of test plants, with observations every 2 to 4 weeks.

(6) **Season of application.** The test substance is to be applied over the period of time or season according to the proposed label instructions.

(7) **Test locations.** The pesticide should be tested in those geographic locations where it is expected to be used, as based on proposed label use sites. Where important species diversity and physiographic differences occur within a region of intended application, regional testing may be inadequate, and testing at a more specific region or biome level may be required. United States regional areas of potential testing include Northeastern temperate deciduous, Southeastern temperate deciduous, Northern grassland (prairie), Southern grassland (prairie), Northwestern (and Alaskan) conifer forest and high desert, Southwestern chaparral Mediterranean and low desert, and Hawaiian and Caribbean tropical regions.

(c) **Reporting.** In addition to the information required by OPPTS 850.4000 and 850.4400, the test report should include the test conditions (including soil, water, and environmental conditions) and the determination of the 50 percent detrimental effect level.

(d) **Data reporting.** (1) The registrant's report on aquatic field testing studies should include all information necessary to provide:

(i) A complete and accurate description of the field treatments and procedures.

(ii) Sampling data and phytotoxicity rating.

(iii) Data on storage of the plant material, if so performed.

(iv) Report any chemical analysis of the plant material.

(v) Reporting of the data, rating system and statistical analysis.

(vi) Quality control measures/precautions taken to ensure the fidelity of the operations.

(2) Each aquatic field testing report should include the following information:

(i) **General.** (A) Cooperator or researcher (name and address), test location (county and state; country, if outside of the United States), and date of study.

(B) Name (and signature), title, organization, address, and telephone number of the persons responsible for planning/supervising/monitoring and, for field plot studies, applying the pesticide.

(C) Trial identification number.

(D) Quality assurance measures employed:

(*1*) Control measures/precautions followed to ensure the fidelity of the phytotoxicity determinations.

(*2*) Record-keeping procedures and availability of logbooks.

(*3*) Skill of the laboratory personnel.

(*4*) Equipment status of the laboratory or greenhouse.

(*5*) Degree of adherence to good laboratory practices.

(*6*) Degree of adherence to good agricultural practices in maintaining healthly plants.

(E) Other information the registrant considers appropriate and relevant to provide a complete and thorough description of the test procedures and results.

(ii) **Test substance (pesticide).** (A) Identification of the test pesticide AI including chemical name, common name (ANSI, BSI, ISO, WSSA), and company developmental/experimental name.

(B) Percentage of AI in the end-use product or a representative end-use product from the same major formulation category for that general use pattern.

(C) Additional solvents or adjuvants used to dissolve and apply the pesticide if the pesticide is insoluble in or immiscible with water.

(D) Dose rates in terms of AI per area of land or final concentration in the test waters.

(E) Dose rates in terms of less than the maximum label rate as though it were applied directly to the surface of a 15–cm or 6–in water column or in terms of less than the onefold concentration as tested in Tier I with dosages in a geometrical progression of no more than twofold and with subtoxic (<EC50 level) and nontoxic (no-observable-effect-level) concentrations.

(F) Method of application including equipment type, nozzles, pressure, etc.

(G) Number of applications.

(iii) **Plant species.** (A) Identification of the test aquatic plant species as noted:

(*1*) *Dicotyledonae* (dicots), one family member.

(*2*) *Monocotyledonae* (monocots), three family members.

(*3*) Vascular *Cryptogamae* (ferns and allies), three family members.

(*4*) *Bryophyta* (mosses) or *Hepatophyta* (liverworts), one family member.

(*5*) Algae (including *Cyanophyta*), each division represented (five).

(B) Identification of the cultivars of the plant species used and source where available.

(C) Identification of the number of replicates and the number of plants per replicate per dose.

(D) Identification of the date of the plant addition to the growth media without test chemical (for stabilization of plant growth, if necessary) , date of pesticide application, and date of phytotoxicity rating or harvest and analyses.

(iv) **Site of the test.** (A) Site description of the aquatic field testing study as to the type of systems (enclosed, controlled areas of a lake, pond, swamp, or stream, or artificial water systems such as aquaria, or large tubs).

(B) Location of tbe test sites as to whether the test was performed in the following general geographical regions in which the pesticide is to be used: Northeastern temperate deciduous, Southeastern temperate deciduous, Northern grassland (cool prairie), Southern grassland (warm prairie), Northwestern (and Alaskan) conifer forest and high desert, Southwestern chaparral Mediterranean and low desert, Hawaiian and Caribbean semi-tropical and tropical regions.

(C) Climatological data during the test (records of applicable conditions for the type of site, i.e., temperatures, thermoperiods, rainfall or water regime, light regime—intensity and quality, relative humidity, wind speed, etc.).

(D) Physical environment characteristics such as tidal action, water turbidity, flow rates, salinity, and degree of exposure of the environment.

(E) Substrate characteristics (type of growth media including its physical and chemical properties, including pH) including soil type of bottom muds.

(v) **Results.** (A) Phytotoxicity rating (including a description of the rating system) for each plant or plant population (individual container) in the test.

(B) Weight, size (vascular plants) or other growth parameters that may have been measured to ascertain toxic effects of the pesticide upon the plants.

(C) Statistical analysis of the results including environmental or effective concentration (EC) values.

(vi) **Evaluation.** (i) Determination as to whether further aquatic field testing with aquatic species is necessary.

(e) **References.** The following references should be consulted for additional background material on this test guideline.

(1) Davis, J.A. Comparison of static-replacement and flow-through bioassays using duckweed, *Lemna gibba* G-3. EPA Report No. EPA 560/ 6-81-003 (1981).

(2) Holst, R.W. et al. Effect of several pesticides on the growth and nitrogen assimilation of *Azolla-Anabaena* symbiosis. *Weed Science* 30:54–58 (1982).

(3) Little, T.M., and F.J. Hills. Agricultural Experimentation—Design and Analysis. Wiley, NY (1978).

(4) Sculthorpe, C.D. The Biology of Aquatic Vascular Plants. London. Arnold Publishers (1967).

(5) Truelove, D. Research Methods in Weed Science, 2nd Ed., Southern Weed Science Society, Auburn Printing Inc., Auburn, AL (1977).

(6) Voshell, J.R. Jr. Using Mesocosms to Assess the Aquatic Ecological Risk of Pesticides: Theory and Practice. Misccellaneous Publication No. 75, 34th Annual Meeting of the Entomological Society of America, Nov. 29–Dec. 3, 1987MPPEAL 75: 1-88 (1987).

(7) Touart, L. W. and M. W. Slimak. Mesocosm Approach for Assessing the Ecological Risk of Pesticides. Miscellaneous Publication No. 75, 34th Annual Meeting of the Entomological Society of America, Nov. 29—Dec. 3, 1987. MPPEAL 75: 1-88 (1987).

(8) DeNoyelles, F. Jr. et al. Use of Experimental Ponds to Assess the Effects of A Pesticide on the Aquatic Environment. Miscellaneous Publication No. 75, 34th Annual Meeting of the Entomological Society of America, Nov. 29 - Dec. 3, 1987. MPPEAL 75: 1-88 (1987).

(9) Siefert, R. E. et al. Littoral Enclosures for Aquatic Field Testing of Pesticides: Effects of Chlorpyrifos on a Natural System. Miscellaneous Publication No. 75, 34th Annual Meeting of the Entomological Society of America, Nov. 29—Dec. 3, l987. MPPEAL 75: 1-88 (1987).